Revista Internacional de **BOTANICA** EXPERIMENTAL **ΦYTON** International Journal of EXPERIMENTAL **BOTANY**

Fundada en 1951 por Founded 1951 by

Miguel Raggio & Nora Moro de Raggio

Editor Fundador: Dr. Miguel Raggio | Editor Ejecutivo: Dr. Carlos A. Busso

FUNDACIÓN ROMULO RAGGIO

Gaspar Campos 861, 1638 Vicente López (BA), Argentina

55º ANIVERSARIO (2006) 75: 103-108 55th ANNIVERSARY

Variability in accumulation of free proline on *in vitro* calli of four bean (*Phaseolus vulgaris L.*) varieties exposed to salinity and induced moisture stress

(With 2 Tables & 4 Figures)

Variabilidad en la acumulación de prolina libre en callos in vitro de cuatro variedades de soja expuestas a salinidad y estrés hídrico inducido

(Con 2 Tablas y 4 Figuras)

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Abstract. This paper reports the genotypic variability in the accumulation of proline on the *in vitro* calli of bean cultivars exposed to induced water and salinity stress. Remarkable variations in the proline content were found among bean cultivars exposed to both stress factors.

Key words: Proline, *in vitro* calli, *Phaseolus vulgaris*, bean.

Resumen. Este trabajo informa la variabilidad genotípica en la acumulación de prolina en callos *in vitro* de frijol expuestos a estrés hídrico y salino. Se encontraron variaciones notables en el contenido de prolina de los cultivos sometidos a ambos tipos de estrés.

Palabras clave: Prolina, callo *in vitro*, *Phaseolus vulgaris*, frijol.

INTRODUCTION

Bean (*Phaseolus vulgaris* L.) is an important high-protein food crop in Mexico and many Latin American countries of the world. Because of an increasing population, there is a great demand for this food crop, but the production of it is affected by several biotic and abiotic stress factors prevailing in semiarid regions of the world. (Maiti, 1997; Moreno Limon, 1998). The

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[3] This work has been supported by PAICYT CN-150-99

selection of bean cultivars adapted to salinity, drought and other abiotic stresses is a main goal of the breeders. Therefore, several investigations have been directed to study the effect of these stress factors on bean growth. This will contribute to understand the resistance mechanisms involved in different bean cultivars with special reference to drought, salinity, high temperature and low nutrients (Cramer et al., 1988; Mishra et al., 1994; Quintero et al., 1999; Moreno Limón et al., 2000; Nuñez-González et al., 2001).

Proline accumulation is a plant resistance mechanism to various stress factors, such as drought (Naidu et al., 1992; Stewart & Lee, 1974; Singh et al., 1972); salinity (Treichel, 1975), low temperature (Benko, 1986; Chu et al., 1974 and high temperature (Oshanina, 1972). In tolerant genotypes, a high accumulation of proline and little anomaly on chloroplast ultrastructure were observed (Nuñez-Gonzalez et al., 2001). Therefore, the accumulation of proline is considered an indicator for selection of plants tolerant to various stress factors (Singh et al., 1972; Van Rensburg & Kruger, 1994; Flores, 1997). Techniques of *in vitro* tissue culture have been utilized as a valuable tool in the selection of crop cultivars for tolerance to salinity, and for studying the tolerance mechanisms to this stress factor (Revilla & Cañal, 1999; Kim & Song, 1984; Lupotto et al., 1988; Mongodi et al., 1988; Huang-Peiming & Ge-Koulin, 1989; Mahammed et al., 1992). Assays with this technique have demonstrated that increased salinity raised the accumulation of free proline in calli of bean. At the same time, proline content diminished with a decrease in salinity (Broetto et al., 1995; Sawires et al., 1997 cited by Maiti et al., 1997). The objective of the present study was to study the effect of water and salinity stresses on the proline accumulation in cultivars of bean, and to establish its relation to the resistance mechanism of this species to these stress factors.

MATERIALS AND METHODS

Seeds of four varieties of *Phaseolus* cultivars, (Pinto Americano, Pastilla, Flor de Mayo and Flor de Junio) were disinfected in 70% ethanol and sodium hypochlorite (15% v/v) for 10 min. and immediately sown under asceptic conditions on sterilized 0.7% agar under a laminar flow chamber. The explants of 1 cm^2 cotyledonary leaves were obtained from in vitro seedlings. They were sown asceptically in glass vials of 120 mL with approx. 30 mL of MS (Murashige-Skoog, 1962) medium with vitamins, 100 mg.L of mio-inositol, 1.5, 2.0, 3.0, 5.0 and 10 mg.L of 2,4-D (for induction of callus *in vitro*) and agar at 0.7%. The pH was adjusted to 5.7 with NaOH 0.1 N or HCl 0.1 N before addition to agar which was sterilized in a pressure chamber at 121ºC and 15 pound pressure for 15 minutes. The *in vitro* culture was maintained in a photoperiod of 16 h to a temp. of 26±1ºC.

Salt concentrations utilized followed Lupotto et al. (1988) and Moreno Limón (1998). Sections of calli *in vitro* were subcultured in glass of 120 mL with approximately 30 mL of MS culture. Then, solutions of (0.1 and 0.15 M) were added to the main culture for having salinity stress. Each test was compared against its respective control.

Polyethylene glycol (PEG 6000) was used to induce water stress. Sections of calli *in vitro* were subcultured in test tubes (18 x 150mm) in MS medium supported with filter paper. A glass of 120 mL with approximately 30 mL of MS culture and two concentrations of polyethylene glycol (PEG) (10 and 15% which are equivalent to –0.6 and –1 Mpa, respectively) was used for inducing water stress. Each test was compared with its respective control.

Determination of proline. For determination of proline in calli exposed to drought and salinity treatments, we utilized the techniques of Zuñiga et al. (1989) and Bates (1973).

Five g samples of calli were homogenized in 10 mL of aqueous solution of sulfosalicylic acid at 3%. Then the solution was filtered rapidly through a buchner funnel using Whatman filter paper Nº 2. A small volume (2 mL) of the filtrate was thereafter taken to a test tube where added 2mL of ninhydric acid and 2 mL of glacial acetic acid were. This was kept in an incubator at 1000C for one hour. Finally the reaction was completed in an ice bath. Thereafter, 4 mL of toluene were added and contents of the tube were inverted during 20 seconds. After this, toluene phase was sucked using a pipette and it was kept at room temperature to stabilize. Finally, absorbance was determined at 520 nm with a visible light spectrophotometer Turner Sequoia 690. We used a tube with toluene as control for calibration of the apparatus.

The concentration of proline was determined from the calibration curve and calcutated by adopting the following equation (ppm of proline from the curve) X volume of aforation / Fresh material, g = ppm proline in tissue.

RESULTS AND DISCUSSION

Determination of free proline on *in vitro* calli exposed to salinity stress. The results obtained from the analysis of variance indicated that there were highly significant differences (P< 0.01) in proline contents among varieties and treatments (Table 1).

Table 1. Analysis of variance of free proline on in vitro calli exposed to salinity stress.

Element	Variation Source	DF	MSS	F-value
Proline	Variety	3	0.030	12.37 **
	Treatment	2	0.012	5.13 **

** Highly significant (P<0.01).

Pinto americano showed higher (p<0.01) free proline contents than the other varieties (Fig. 1). Flor de Mayo and Flor de Junio had the lowest (p<0.01) contents. Free proline content was lowest (p<0.01) at 0.15 M NaCl compared with the control (Fig. 2). This is in contrast to the report by Sawires et al. (1997), cited by Maiti et al. (2000) who reported that free proline in general increased with an increase in salinity. Sivaramkrishnan et al. (1988) and Revilla & Cañal (1999) also reported that salinity stress in calli of olive showed an increase in proline under salinity stress. Our results need to be verified in further studies.

Determination of free proline on *in vitro* calli exposed to drought stress with PEG. Free proline contents of all 4 bean varieties were significantly different among each other (p<0.01), (Table 2 and Fig. 3). On the other hand, the lower the water potential (15% PEG), the higher (p<0.01) the free proline content (Fig. 4). This result is coincident with findings of Sivaramkrishnan et al. (1988) who reported that an increase in proline was related with a decrease in leaf water potential.

Table 2. Analysis of variance of free proline on *in vitro* calli exposed to induced moisture stress.

Element	Variation Souce	DF	MSS	F-value
Proline	Variety	3	0.036	66.47**
	Treatment	2	0.012	23.87**

** Highly significant (P<0.01).

CONCLUSIONS

A higher free proline content in varieties reported as tolerant (pinto americano and pastilla), compared to the other two varieties indicates that this aminoacid may be highly involved as a plant adaptation mechanism to water and salinity stresses. Proline may act as a osmoregulator compound to equilibrate the osmotic potential in bean cells.

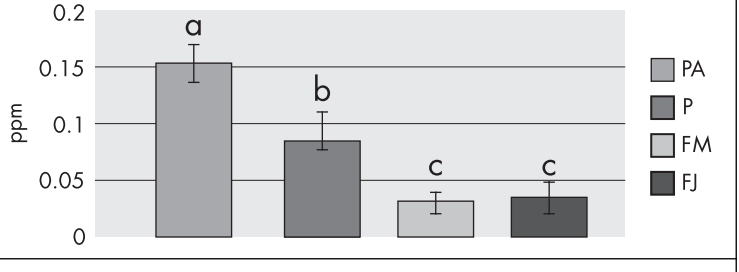

Fig. 1. Free proline content in cotiledonary leaf on *in* vitro callus from 4 var.of *Phaseolus vulgaris* L. under salinity stress.

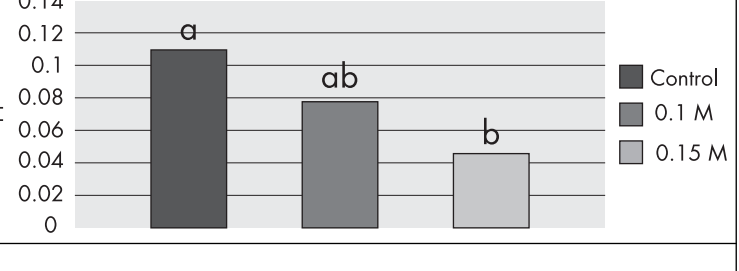

Fig. 2. Free proline content in cotiledonary leaf on *in vitro* callus from 4 var.of *Phaseolus vulgaris* L. in the different salinity treatments with NaCl.

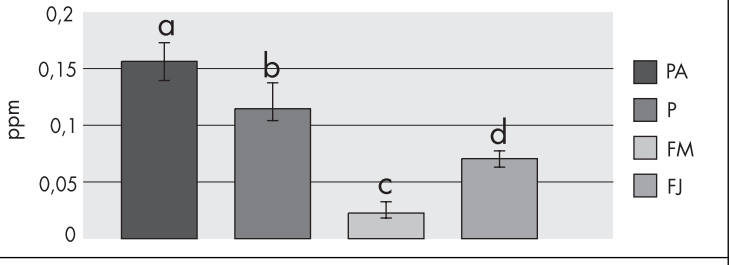

Fig. 3. Free proline content in cotiledonary leaf on *in vitro* callus from 4 var.of *Phaseolus vulgaris* L. under drought stress with PEG.

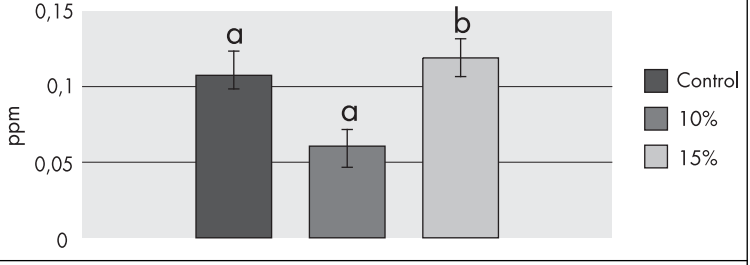

Fig. 4. Free proline content in cotiledonary leaf on *in vitro* callus from 4 var.of *Phaseolus vulgaris* L under various drougth tratments with PEG.

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